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# Transient Response of Amplified Metallothionein Genes in CHO Cells to Induction by Alpha Interferon

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**Alpha interferon treatment of CHO cells elicits the rapid synthesis of many gene products, including metallothionein (MT), a protein which avidly binds heavy metals such as zinc, cadmium, and copper. Since MTs appear to have a pleiotropic role in the cell, ranging from metal detoxification to free-radical scavenging, interferon treatment may trigger a generalized defense mechanism. Activation by interferon, however, was transient, with MT mRNA being maximally detectable by a cytodot procedure within the first hour. Subsequent addition of interferon was ineffective until 7 h after the initial treatment. The action of zinc, a potent inducer of MT, however, remained independent of alpha interferon induction. The transient nature of induction by interferon was examined for altered rate of MT mRNA turnover.**

Physiological responses to interferon exposure, such as establishment of an antiviral state and inhibition of cell growth, correlate with the induction of at least 12 proteins (7, 22, 34). These interferon-mediated events require new mRNA synthesis (6, 29, 31). Although the mechanism of transcriptional regulation by interferon is not well understood, it is clear that the response to interferon is rapid; at least three genes achieve maximum transcription rates within 2 h of exposure (12, 21). In human fibroblasts, the induction of IND-1 and IND-2 genes by interferon is followed by a significant decline in the transcription rate of IND genes and accompanied by an inability of further interferon exposure to reinduce transcription (21). This negative regulatory event, termed desensitization, apparently requires the synthesis of highly labile proteins.

Cadmium resistant ($Cd^r$) CHO cell lines which have amplified metallothionein (MT) genes have provided a sensitive system to examine MT gene-inductive processes (15, 16). Since MT genes have been shown to be activated during inflammatory and acute-phase reactions (18), the possibility that alpha interferon would transcriptionally activate MT genes in $Cd^r$ CHO cells was investigated. In this communication, we present evidence that human alpha interferon rapidly induces the coordinate accumulation of both MT I and MT II mRNA in $Cd^r$ CHO mutant cells which have amplified MT genes (8). The accumulation is transient; maximal MT mRNA concentrations were detected 1 h after exposure. Subsequent interferon treatments 7 h after the initial exposure reinduced MT mRNA accumulation to maximal levels. We also demonstrate that interferon pretreatment did not affect the transcriptional response of these cells to zinc, an extremely effective inducer of MT genes. Concurrent interferon and zinc treatments yielded an additive accumulation of MT mRNA, which suggests that these MT gene inducers activate transcription by different mechanisms.

## MATERIALS AND METHODS

**Materials.** The $Cd^r$ CHO cell line 458-3 was created by ethylmethane sulfate mutagenesis of wild-type CHO cells, followed by stepwise selection on increasing concentrations of cadmium (8). The cells were routinely grown on alpha minimal essential medium with 5% fetal calf serum.

Lyophilized human alpha interferon was purchased from Sigma Chemical Co. and reconstituted as suggested by the manufacturer. Plasmids containing MT (15) and alpha-tubulin (9) cDNA were kindly provided by the Los Alamos Genetics Group and Don Cleveland, respectively.

**Cytodot assay for quantitating RNA accumulation.** The cytodot protocol used was developed by White and Bancroft (35). Briefly, the cytodot procedure involves treatment of induced cells with 5% Nonidet P-40, separation of cytoplasmic extracts from nuclei, and denaturation of cytoplasmic RNA with formaldehyde at 60°C. In most experiments, equivalent samples of cytoplasmic extract were spotted on three filters. One filter was hybridized with a single-stranded primer-extended MT-II probe, another was hybridized to a single-stranded primer-extended MT-I probe, and a control filter was treated with a nick-translated human alpha-tubulin probe. The filters were prehybridized for 12 to 24 h at 65°C in a solution containing 5× SSPE (1× SSPE is 0.15 M NaCl, 10 mM $NaH_2PO_4$, pH 7.7, and 1 mM EDTA), 5× Denhardt solution, 0.1% sodium dodecyl sulfate (SDS), and 100 μg of salmon sperm DNA per ml and hybridized for 48 h at 65°C in an identical solution with $8 \times 10^5$ cpm of MT probe. Subsequently, these filters were washed at 25°C in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS for 30 min and then at 65°C in 0.1× SSC–0.1% SDS for 1 h. The MT-I- and MT-II-probed filters were washed for an additional 2 h at very high stringency (0.01× SSC–0.1% SDS, 65°C), which prohibits cross-hybridization.

To quantitate RNA dots, the dots were localized by autoradiography, placed in scintillation fluid, and counted. Because of the greater length of the MT-I insert and its slightly different base composition, the specific activity of primer-extended MT-I probes was 1.5-fold higher than that of MT-II probes. The molar ratio of MT-II- to MT-I-hybridizing RNA can be computed by the following formula: MT-II/MT-I = 1.5 × cpm of MT-II/cpm of MT-I. MT mRNA levels of three induced samples were quantitated for each experimental point.

**Northern blots of interferon-induced RNA.** Cytoplasmic RNA was isolated from untreated, interferon-induced, and

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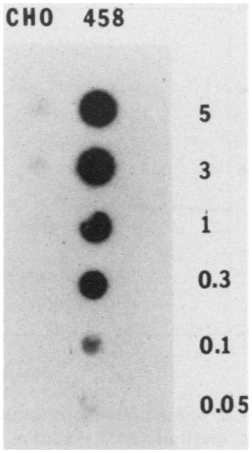


FIG. 1. Analysis of MT gene copy number in Cd$^r$ 458-3 cells. (A) High-molecular-weight DNA isolated from wild-type CHO and Cd$^r$ 458-3 cells was digested to completion with *Hin*cII, serially diluted, denatured, and spotted on nitrocellulose. The filter was then hybridized to an MT-II probe under standard conditions. DNA concentrations (shown to the right, in micrograms) were determined by $A_{260}$ measurements and diphenylamine assays.

zinc-induced Cd$^r$ 458-3 cells by the procedure described by Maniatis et al. (23). The interferon-induced cells were induced for 1 or 3 h with 100 U of interferon per ml. The zinc-induced cells were exposed to 100 μM zinc for 24 h. Ten micrograms of RNA was denatured with glyoxal, loaded on a 1.4% agarose gel, and run at 40 V for 3.5 h in 0.01 M $NaH_2PO_4$, pH 7.0. The glyoxalated RNA was transferred immediately after electrophoresis to nitrocellulose. The hybridization protocols were described above.

**Preparation of primer-extended single-stranded MT probes.** Cloning of CHO MT cDNAs into M13mp9 recombinant phage has been described elsewhere (26). Primer-extended probe synthesis is essentially a scaled-up adenine-sequencing reaction. Briefly, M13 sequencing primer (Bethesda Research Laboratories) was annealed to M13-MT single-stranded template, primer-extended in the presence of [α-$^{32}$P]dATP, chased with 0.5 M dATP, and digested with *Pst*I. After ethanol precipitation, the sample was run on a 1% agarose denaturing gel which had been prepared in 30 mM NaOH and 10 mM EDTA. The single-stranded labeled MT insert was visualized by autoradiography and recovered by electroelution. MT primer-extended probe synthesis yielded $5 \times 10^6$ to $10 \times 10^6$ cpm of single-stranded insert at about $5 \times 10^7$ to $7 \times 10^7$ cpm/μg.

**Nuclear runoff assay.** Nuclear runoff assays were performed as described by Marzluff et al. (24). The labeled RNAs were hybridized as described above to nitrocellulose filters containing 10-μg dots of an MT-II cDNA plasmid and a plasmid with the alpha-tubulin gene. The extent of hybridization was determined by scintillation counting. Typically, about 600 to 800 counts/10 min of labeled RNA from induced cells hybridized to the MT-II plasmid dots. The relative induced rate of MT mRNA transcription was calculated by dividing the MT/tubulin transcription ratio after interferon induction by the MT/tubulin ratio before treatment.

**Pulse polarographic quantitation of CHO MT.** Pulse polarography is an electrochemical technique which is useful for quantitating cysteine-rich proteins such as MT. The pulse polarographic assay was carried out with a Metrohm 626 polarograph as described previously (25). The CHO MT concentrations were estimated from rat MT-I standard polarographic response curves.

## RESULTS

**Amplification of MT genes in the cadmium-resistant 458-3 cell line.** Previously, we isolated a Cd$^r$ CHO cell line, 458-3, which is 100-fold more resistant than wild-type cells to the growth-inhibitory and cytotoxic effects of cadmium (8). We have also shown that this mutant cell line overproduces MT in response to heavy-metal challenge (P. C. Huang, K. Danielson, C. Wong, S. Morris, R. Pine, and P. Bohdan, Fed. Proc. **42**:1916, 1983). In the present study, DNA dot blots were used to estimate the MT gene number in mutant and wild-type cells. We found that 50 ng of mutant DNA and 3 μg of wild-type DNA bound equivalent amounts of CHO MT-II probe (Fig. 1). The same dot blots probed with the CHO MT-I probe also yielded a 60-fold hybridization enrichment for 458-3 DNA (data not shown). These data indicate that both MT genes are amplified in this Cd$^r$ CHO cell line.

**Coordinate induction of 458-3 MT genes by zinc.** Estimates of MT mRNA levels in 458-3 cells induced with zinc were derived from molecular hybridization studies with primer-extended CHO cDNA probes. Treatment with 100 μM zinc increased MT RNA concentrations about 20-fold over constitutive levels (Fig. 2). At this zinc concentration, accumulation of MT RNA was maximal at 30 h. After 30 h, MT RNA concentrations decreased greatly, reaching only 40% of the peak values at 40 and 48 h (Fig. 2A and B). To determine whether both MT mRNA species were being transcribed in response to zinc treatment, RNA from cells induced for 24 h was hybridized to either the MT-I or MT-II probe under highly stringent conditions, which prohibit cross-hybridization. Parallel results from dot blots indicated that heavy

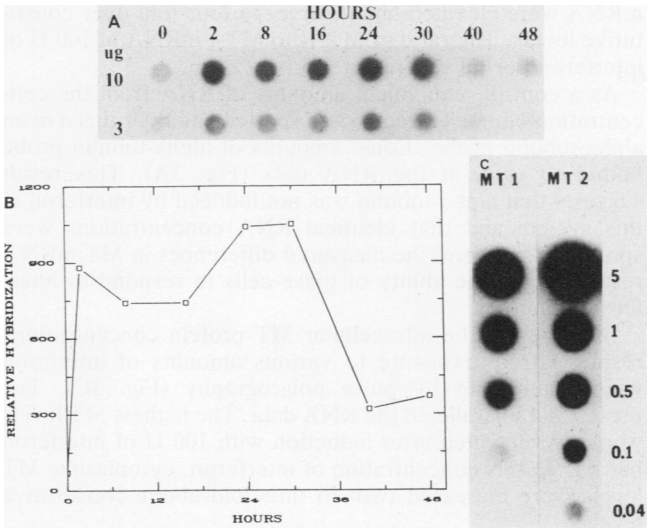


FIG. 2. Induction of MT mRNA in 458-3 cells by zinc. (A) CHO Cd$^r$ 458-3 cells were induced with 100 μM zinc for the indicated times. Cytoplasmic extracts prepared by the cytodot protocol were hybridized to an MT-II probe under high-stringency conditions as described in the text. Each sample contained either 10 or 3 μg of protein. (B) The 10-μg dots shown in panel A were removed, dissolved in Beta Fluor, and counted. The scintillation data were then plotted. (C) Cytoplasmic RNA (concentrations shown to the right, in micrograms) isolated from 458-3 cells induced with 100 μM zinc for 24 h was spotted on nitrocellulose saturated with 20× SSC and hybridized to either an MT-I or MT-II probe under high-stringency conditions.

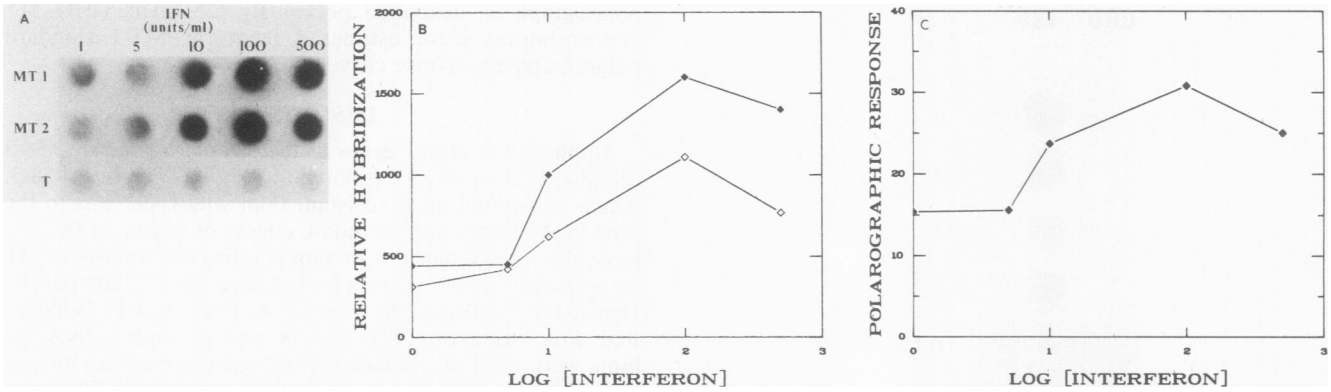


FIG. 3. Effect of interferon concentration on MT mRNA and MT accumulation. (A) CHO Cd$^r$ 458-3 cells were induced for 2 h with the indicated amounts of interferon (IFN). Cytoplasmic extracts were prepared by the cytodot method (35). Portions of cellular extracts representing 10 μg of protein were spotted on three nitrocellulose filters and hybridized to either MT-I, MT-II, or alpha-tubulin (T) probes as described in the text. The autoradiograms of MT-I- and MT-II-probed filters were exposed for 24 h, while the alpha-tubulin filters required 3 to 5 days of exposure. (B) The RNA dots shown in panel A were removed and dissolved in Beta Fluor and their radioactivity was counted for 10 min. The MT-I (◇) and MT-II (◆) counts were normalized to equimolar counts as described in the text. Each point represents the average value for three induced samples. The standard deviations were usually ±5 to 10%. (C) Cells were induced and cytoplasmic extracts were prepared as described for panel A. Typically, 25 μl of extract was assayed by pulse polarography. The polarographic response units are micrograms of MT per milligram of total protein in the extracts.

metals activated transcription of both MT genes (Fig. 2C); the MT-II/MT-I mRNA ratio after induction was 2.5.

**Concentration dependence of interferon induction.** Cd$^r$ CHO cells were exposed for 2 h to increasing concentrations of interferon. MT mRNA accumulation was then quantitated by the cytodot protocol (35). Interferon induced both the MT-I and MT-II genes in these cells (Fig. 3A and B). The inductive response was dose dependent; 100 U of interferon per ml yielded maximal MT mRNA accumulation. At this interferon conentration, the levels of MT-II and MT-I mRNA were elevated about three- to four-fold over constitutive levels. The ratio of MT-II to MT-I mRNA at 100 U of interferon per ml was about 1.5.

As a control, equivalent amounts of RNA from the concentration-course extracts were spotted and hybridized to an alpha-tubulin probe. Equal amounts of alpha-tubulin probe bound to each of the RNA dots (Fig. 3A). This result suggests that alpha-tubulin was not induced by interferon in this system and that identical RNA concentrations were spotted. Therefore, the measured differences in MT mRNA resulted from the ability of these cells to respond to interferon induction.

Increases in the intracellular MT protein concentrations resulting from exposure to various amounts of interferon were determined by pulse polarography (Fig. 3C). The protein data paralleled the RNA data. The highest MT levels were also detected after induction with 100 U of interferon per ml. At this concentration of interferon, cytoplasmic MT levels were increased two- to threefold above constitutive levels.

**Time course of interferon induction.** Interferon rapidly induced MT mRNA accumulation in Cd$^r$ CHO cells (Fig. 4). Twenty to forty minutes after interferon treatment, increased concentrations of MT-I and MT-II mRNA were measurable. However, the accumulation was transient; a maximal fourfold increase in MT mRNA levels relative to noninduced cells was obtained 1 h postinduction. In contrast, exposure to zinc elevated MT mRNA levels 20-fold (Fig. 2B). Furthermore, the zinc response was not transient; maximal MT mRNA concentrations after zinc induction were detected after 24 to 30 h.

Nuclear runoff assays were performed to ascertain whether the interferon-induced transient increases in MT mRNA accumulation resulted from elevated rates of MT mRNA transcription (Fig. 5). Thirty minutes after interferon treatment, the MT mRNA transcription rate was about threefold higher than in noninduced controls. Subsequently, the rate of transcription from MT genes decreased. At 4 h postinduction, MT mRNA transcription had returned to control levels.

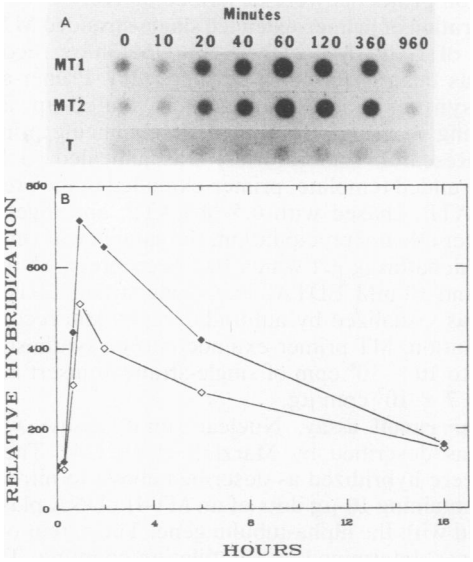


FIG. 4. Time course of interferon induction of MT-I and MT-II mRNA accumulation. (A) 458-3 cells were induced for various time periods with interferon (100 U/ml). Cytoplasmic extracts were prepared by the cytodot protocol. Equivalent samples of cellular extracts representing 10 μg of protein were spotted on three nitrocellulose filters and hybridized to either MT-I, MT-II, or alpha-tubulin (T) probes as described in the legend to Fig. 3. (B) The RNA dots shown in panel A were removed, dissolved in Beta Fluor, and counted for 10 min. The MT-I (◇) and MT-II (◆) counts were normalized to equimolar counts as described in the text.

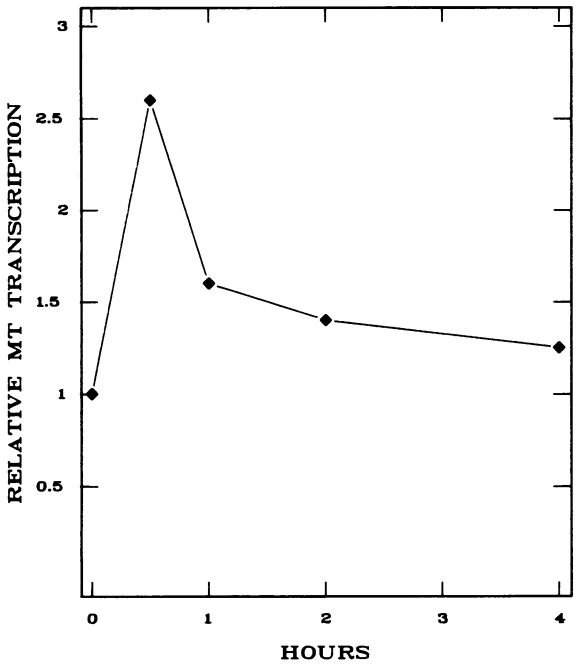


FIG. 5. Induction of MT mRNA transcription by interferon. The rate of MT mRNA transcription was assayed 0, 0.5, 1, 2, and 4 h after induction with interferon (100 U/ml) by nuclear runoff procedures as described in the text. The relative MT mRNA transcription rate was calculated by the MT/tubulin ratio.

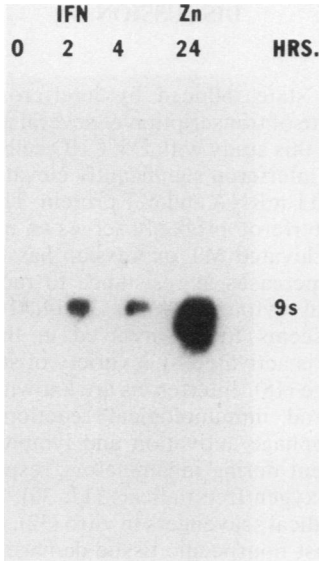


FIG. 6. Northern blot of RNA isolated from interferon- and zinc-induced 458-3 cells. Ten micrograms of cytoplasmic RNA extracted from 458-3 cells that were noninduced (lane 0), induced with interferon (IFN) (100 U/ml) for 2 h (lane 2) or 4 h (lane 4), or induced for 24 h with 100 μM zinc were denatured with glyoxal and dimethyl sulfoxide and electrophoresed through a 1.4% agarose gel in 10 mM $NaH_2PO_4$ (pH 7.0). The glyoxalated RNA was transferred immediately to nitrocellulose and hybridized to an MT-II probe under conditions described in the text. This autoradiogram was exposed at −70°C for 72 h. The position of 9S mRNA is shown.

**Northern blots of interferon-induced RNA.** RNA isolated from control, interferon-, and zinc-treated cells were compared by Northern blotting procedures (Fig. 6). Results from the Northern analysis, like those obtained with cytodot assays, indicated that interferon induced MT mRNA in CHO $Cd^r$ cells. The level of induction of MT genes by interferon was severalfold lower than the maximal inductive response to zinc. Interferon induced 9S MT mRNA species which comigrated with constitutive and zinc-induced MT mRNAs. No truncated or aberrant MT mRNA was detectable after interferon exposure.

**Reinduction of MT mRNA accumulation by a second exposure to interferon.** In human fibroblasts, continuous exposure to interferon can result in the inability of further interferon treatment to reinduce those genes that are normally responsive to interferon (21). We examined this desensitization phenomenon in our system. A second dose of 100 U of interferon per ml was applied to cells previously exposed to the same concentration of interferon for 2, 7, and 15 h. Accumulation of MT mRNA was measured 1 h after the second treatment by the cytodot protocol. At 2 h after the initial exposure to interferon, the second interferon treatment only slightly increased MT mRNA accumulation (Table 1). However, at 7 and 15 h, added interferon increased MT mRNA concentrations two- to threefold.

**Effect of interferon treatment on induction of MT genes by zinc.** Heavy metals such as zinc and cadmium rapidly induce transcription from MT genes in cultured cells (10, 11, 15, 36). Since interferon also elicits MT expression, we examined whether interferon preexposure influences responsiveness to zinc in $Cd^r$ CHO cells. The results (Table 2) suggested that treatment with interferon did not affect the extent of MT mRNA accumulation attributable to the subsequent addition of zinc. Comparison of the differences in hybridization between cytoplasmic extracts of cells treated with zinc and interferon and cells challenged only with zinc indicated that approximately equivalent amounts of MT mRNA were induced by zinc whether or not the cells had been pretreated with interferon. Exposure to 100 U of interferon per ml for 2, 7, or 15 h apparently did not influence the ability of these cells to respond to zinc (50 μM). Furthermore, the interferon and zinc responses were additive. The extent of MT mRNA accumulation in 458-3 cells induced simultaneously with interferon and zinc was approximately equal to the aggregate MT mRNA levels in cells induced separately. 458-3 cells treated with interferon prior to zinc application also yielded more MT mRNA than cells treated with either of these inducers alone.

TABLE 1. Reinduction of MT mRNA accumulation by a second exposure to interferon[a]

| Interferon treatment (h) | Relative MT mRNA levels[b] | Relative hybridization increase[c] |
|---|---|---|
| 1 | 520 ± 68 | |
| 2 | 497 ± 70 | |
| 2 + 1 | 527 ± 74 | 1.06 |
| 7 | 167 ± 24 | |
| 7 + 1 | 463 ± 26 | 2.61 |
| 15 | 220 ± 39 | |
| 15 + 1 | 505 ± 40 | 2.30 |

[a] 458-3 cells were preexposed to interferon (100 U/ml) for 2, 7, or 15 h before a second 1-h interferon (100 U/ml) treatment. MT-II RNA accumulation was quantitated 1 h later by the cytodot protocol. Control samples were exposed to the same interferon concentration for 1, 2, 7, or 15 h prior to being assayed for MT mRNA accumulation. Each value represents the average of three replicates.

[b] Difference between experimental and constitutive hybridization values.

[c] Determined by dividing second-exposure hybridization values by their corresponding controls.

## DISCUSSION

The antiviral state induced by interferon involves increases in the rate of transcription of several genes. We have demonstrated in this study with $Cd^r$ CHO cells that exposure to human alpha interferon significantly elevated the concentration of both MT mRNA and MT protein. This induction of MT genes by interferon probably serves an important physiological role. Elevated MT expression has been shown to correlate with increases in resistance to radiation damage and heavy-metal cytotoxicity (2, 3, 14, 16, 18, 19). In addition, MT seems to be involved in the acute-phase response which is activated by a variety of stresses, including tissue damage (18). Interferons are known to affect many inflammatory and immunological reactions. Interferon-mediated macrophage activation and lymphocyte cytotoxicity enhancement during inflammatory responses result in the release of oxygen free radicals (17, 30). Since MTs are effective free-radical scavengers in vitro (32), they may serve to protect against nonspecific tissue damage resulting from high free-radical concentrations generated by interferon-activated macrophages or cytotoxic lymphocytes.

Mammals synthesize two isospecies of MT, MT-I and MT-II. In the mouse, both isogenes are induced to the same extent by heavy metals and glucocorticoids (28). In humans, however, specific isogene expression is tissue specific and regulated differentially by either of the two classes of inducers mentioned above (27, 33). Recently, Friedman et al. have demonstrated that the MT-II gene of a human neuroblastoma cell line is responsive to interferon (12, 13). In our study, both CHO MT isogenes were shown to be inducible by both zinc and interferon treatment. Induction with 100 μM zinc yielded an MT-II/MT-I mRNA ratio of 2.5. At 100 U of interferon per ml the ratio of MT-II to MT-I mRNA was 1.5. This result indicates that either these isogenes respond differently to these inducers or that their rates of RNA decay are different.

Our study also showed that zinc was more effective than interferon as an inducer of MT mRNA accumulation in CHO $Cd^r$ cells. Interferon maximally increased MT mRNA levels by fourfold, while zinc yielded a 20-fold increase in this $Cd^r$ cell line. Interferon induction is probably initiated by its binding to specific receptors in the plasma membrane (1, 4). Receptor saturation may thus dictate the maximum transcriptional response and explain the limited elevation of MT levels observed. Heavy metals can enter cells by passive diffusion (8). Diffusion events are usually more susceptible to saturation than receptor-mediated processes. Therefore, the differential inducibility of MT genes may reflect, in part, the differential uptake of heavy metals and interferon.

Increased MT mRNA transcription was detectable minutes after interferon exposure in this CHO cell line, and maximum MT mRNA concentrations were achieved in about 1 h (Fig. 4). Rapid accumulation of other interferon-induced RNAs has been described previously (7, 12, 21). The maximum transcription rate of several of these genes occurs 5 to 30 min after interferon treatment (12, 21). Although a rapid interferon-mediated response may be expected, the limited duration of the inductive response remains unexplained, because an elevated response to zinc persisted for at least 30 h (Fig. 2). Control experiments have shown that interferon remained active in the medium for at least 3 h. Thus, the transient interferon-induced response probably does not result solely from inducer consumption or instability but may involve other factors, such as receptor saturation or interferon-inducible negative regulatory molecules.

TABLE 2. Effect of interferon treatment on induction of MT genes by zinc[a]

| Interferon pretreatment (h) | Subsequent zinc treatment (h) | Relative MT mRNA level[b] | Induction due to zinc[c] |
|---|---|---|---|
| 0 | 0 | 65 ± 30 | |
| | 2 | 1,218 ± 230 | 1,153 |
| 2 | 0 | 497 ± 70 | |
| | 2 | 1,696 ± 164 | 1,199 |
| 7 | 0 | 167 ± 24 | |
| | 2 | 1,950 ± 49 | 1,783 |
| 15 | 0 | 220 ± 39 | |
| | 2 | 1,590 ± 195 | 1,370 |

[a] 458-3 cells were preexposed to interferon (100 U/ml) for 0, 2, 7, or 15 h before treatment with 50 μM zinc. MT mRNA accumulation was quantitated 2 h later by the cytodot protocol. Interferon control samples were exposed to interferon (100 U/ml) for 2, 7, or 15 h prior to being assayed for MT mRNA accumulation.

[b] Difference between experimental and constitutive hybridization values. Simultaneous treatment with interferon (100 U/ml) and zinc (50 μM) for 2 h yielded relative MT mRNA 1,813 ± 20, a net gain over interferon treatment alone of 1,316. Each value represents the average of three replicates ± standard deviation.

[c] Difference between samples with zinc added and their corresponding interferon controls.

The half-life of zinc-induced MT mRNA is about 12 h in CHO cells (unpublished results). Given the stability of MT mRNA, the significantly decreased accumulation only hours after treatment suggests that interferon-induced posttranscriptional events may reduce accumulation by increasing RNA turnover. However, preexposure to interferon did not affect the transcriptional response to zinc. If interferon affects zinc-induced MT mRNA stability, a decreased accumulation of MT mRNA at 2 h would be expected. Treatment with zinc generated equivalent amounts of MT mRNA whether or not the cells had been preexposed to interferon (Table 2). Apparently, exposure to interferon does not affect the extent of zinc-induced MT mRNA accumulation in 458-3 cells.

The additive induction response to zinc at saturating interferon concentrations (Table 2) argues that interferon and zinc are independently activating the transcription of MT genes by different inductive mechanisms. This is expected, since multiple inducer-responsive sequences exist in the regulatory region of MT genes (5, 20, 33). The differential responses of MT genes to widely varying inducing agents suggests pleiotropic functions for MT. While MT is almost certainly involved in metal homeostasis, it may play a larger role in general defense mechanisms important in cellular responses to environmental stress. Further studies characterizing MT gene structure, MT gene expression, and the properties of the MT protein will be required to completely elucidate the biochemical roles for MT.

## ACKNOWLEDGMENT

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## LITERATURE CITED

1. **Anderson, P., Y. K. Yip, and J. Vilcek.** 1982. Specific binding of $^{125}I$ human interferon-gamma to high affinity receptors on human fibroblasts. J. Biol. Chem. **257**:1301–11304.
2. **Bakka, A., A. S. Johnson, L. Enderssen, and H. E. Rugstad.** 1982. Radioresistance in cells with high content of metallothionein. Experientia **38**:381–383.

3. **Beach, L. R., and R. D. Palmiter.** 1981. Amplification of the metallothionein-I gene in cadmium resistant mouse cells. Proc. Natl. Acad. Sci. USA **78**:2110–2114.
4. **Branca, A. A., C. R. Faltynek, S. B. D'Alessandro, and C. Baglioni.** 1982. Interaction of interferon with cellular receptors. J. Biol. Chem. **257**:13291–13296.
5. **Carter, A. D., B. K. Felber, M. J. Walling, M. F. Jubier, C. J. Schmitt, and D. H. Hamer.** 1984. Duplicated heavy metal control sequences of the mouse metallothionein-I gene. Proc. Natl. Acad. Sci. USA **81**:7392–7396.
6. **Colonna, R. J.** 1981. Accumulation of newly synthesized mRNAs in response to human fibroblast beta interferon. Proc. Natl. Acad. Sci. USA **78**:4763–4766.
7. **Colonna, R. J., and R. H. L. Pang.** 1982. Induction of unique mRNAs by human interferons. J. Biol. Chem. **257**:9234–9237.
8. **Corrigan, A. J., and P. C. Huang.** 1983. Cadmium and zinc flux in wild-type and cadmium-resistant CHO cells. Biol. Trace Element Res. **5**:25–33.
9. **Cowan, N. J., P. R. Dobner, E. V. Fuchs, and D. W. Cleveland.** 1983. Expression of human alpha-tubulin genes: interspecies conservation of 3′ untranslated regions. Mol. Cell. Biol. **3**:1738–1745.
10. **Durnam, D. M., J. S. Hoffman, C. J. Quaiffe, E. P. Benditt, H. Y. Chen, R. L. Brinster, and R. D. Palmiter.** 1984. Induction of mouse metallothionein-I mRNA by bacterial endotoxin is independent of metals and glucocorticoid hormones. Proc. Natl. Acad. Sci. USA **81**:1053–1056.
11. **Durnam, D. M., and R. D. Palmiter.** 1981. Transcriptional regulation of the mouse metallothionein-I gene by heavy metals. J. Biol. Chem. **256**:5712–5716.
12. **Friedman, R. L., S. P. Manly, M. McMahon, I. M. Kerr, and G. R. Stark.** 1984. Transcriptional and posttranscriptional regulation of interferon-induced gene expression in human cells. Cell **38**:745–755.
13. **Friedman, R. L., and G. R. Stark.** 1985. Alpha-interferon-induced transcription of HLA and metallothionein genes containing homologous upstream sequences. Nature (London) **314**:637–639.
14. **Gick, G. G., and K. S. McCarty, Sr.** 1982. Amplification of the metallothionein-I gene in cadmium- and zinc-resistant Chinese hamster ovary cells. J. Biol. Chem. **257**:9049–9053.
15. **Griffith, J. K.** 1985. Coordinate expression of amplified metallothionein I and II genes in cadmium-resistant Chinese hamster cells. Mol. Cell. Biol. **5**:3525–3531.
16. **Hildebrand, C. E., R. A. Tobey, E. W. Campbell, and M. D. Enger.** 1979. A cadmium-resistant variant of the Chinese hamster (CHO) cell with increased metallothionein induction capacity. Exp. Cell Res. **124**:237–246.
17. **Johnston, R. B., and J. E. Lehymeyer.** 1976. Elaboration of toxic oxygen by-products by neutrophils in a model of immune complex disease. J. Clin. Invest. **57**:836–841.
18. **Karin, M.** 1985. Metallothionein: proteins in search of function. Cell **41**:9–10.
19. **Karin, M., G. Cathala, and C. Nguyen-Huu.** 1983. Expression and regulation of a human metallothionein gene carried on an autonomously replicating shuttle vector. Proc. Natl. Acad. Sci. USA **80**:4040–4044.
20. **Karin, M., A. Haslinger, H. Holtgreve, R. I. Richards, P. Krauter, H. M. Westphal, and M. Beato.** 1984. Characterization of DNA sequences through which cadmium and glucocorticoid hormones induce human metallothionein-IIA gene. Nature (London) **308**:513–519.
21. **Larner, A. C., A. Chaudhuri, and J. E. Darnell, Jr.** 1986. Transcriptional induction by interferon. J. Biol. Chem. **261**:453–459.
22. **Lengyel, P.** 1982. Biochemistry of interferons and their actions. Annu. Rev. Biochem. **51**:251–282.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
24. **Marzluff, W. F., Jr., E. C. Murphy, Jr., and R.-C. C. Huang.** 1973. Transcription of ribonucleic acid in isolated mouse myeloma nuclei. Biochemistry **12**:3440–3446.
25. **Olafson, R. W., and R. G. Sim.** 1979. An electrochemical approach to quantitation and characterization of metallothioneins. Anal. Biochem. **100**:343–351.
26. **Pine, R., M. Cismowski, S. W. Liu, and P. C. Huang.** 1985. Construction and characterization of a library of metallothionein coding sequence mutants. DNA **4**:115–126.
27. **Richards, R. I., A. Heguy, and M. Karin.** 1984. Structural and functional analysis of the human metallothionein 1A gene: differential induction by metal ions and glucocorticoids. Cell **37**:263–272.
28. **Searle, P. F., B. L. Davison, G. W. Stuart, T. M. Wilkie, G. Norstedt, and R. D. Palmiter.** 1984. Regulation, linkage, and sequence of mouse metallothionein I and II genes. Mol. Cell. Biol. **4**:1221–1230.
29. **Shulman, L., and M. Revel.** 1980. Interferon-dependent induction of mRNA activity for (2′5′) oligoisoadenylate synthetase. Nature (London) **288**:98–100.
30. **Tauber, A. I., and B. M. Babior.** 1977. Evidence of hydroxyl radical production by human neutrophils. J. Clin. Invest. **60**:374–379.
31. **Taylor, J.** 1964. Inhibition of interferon action by actinomycin. Biochem. Biophys. Res. Commun. **14**:447–451.
32. **Thornally, P. J., and M. Vasak.** 1985. Possible role for metallothionein in protection against radiation induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. Biochim. Biophys. Acta **827**:36–44.
33. **Varshney, U., N. Jahroudi, R. Foster, and L. Gedmamu.** 1986. Structure, organization, and regulation of human metallothionein $I_F$ gene: differential and cell-type-specific expression in response to heavy metals and glucocorticoids. Mol. Cell. Biol. **6**:26–37.
34. **Weil, J., C. J. Epstein, L. B. Epstein, J. J. Sedmak, J. L. Sabra, and S. E. Grossberg.** 1983. A unique set of polypeptides induced by gamma interferon in addition to those induced in common with alpha and beta interferons. Nature (London) **301**:437–439.
35. **White, B. A., and F. C. Bancroft.** 1982. Cytoplasmic dot hybridization. J. Biol. Chem. **257**:8569–8572.
36. **Yagle, M. K., and R. D. Palmiter.** 1985. Coordinate regulation of mouse metallothionein I and II genes by heavy metals and glucocorticoids. Mol. Cell. Biol. **5**:291–294.